REGENERATION AND EVALUATION OF RICE GERPLASM ACCESSIONS IN SEED BANK OF CUULONG DELTA RICE RESEARCH INSTITUTE

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ABSTRACT

Annual regeneration and evaluation of local rice accessions stored in seed banks are crucial for genetic resource conservation. Since 2018, a considerable number of local rice accessions in the CLRRI rice seed bank have not been renewed, as a result, these are at risk of being lost. Therefore, the present study was conducted on a population of 1,812 local rice accessions in the seed bank and 941 local Vietnamese rice accessions imported from IRRI to rejuvenate and re-evaluate stored and imported germplasm accessions. The local rice accessions were replanted and evaluated in experimental fields in the 2018 and 2019 Dry Seasons (DS). The results revealed that a total of 2,673 local rice varieties were obtained and stored back in the seed bank. In which two populations of 328 and 903 local rice accessions were re-evaluated based on several traits, the results showed the significant diversity of these two populations. In addition, four local rice accessions were identified with high-quality/long-grain/glutinous round-grain properties that could be promising lines for the breeding program. The results of this study will be efficient the genetic resource conservation and exploitation in the future.

Keywords: Accession, conservation, local rice.

INTRODUCTION

The Vietnam rice resource is well-known to be highly diverse. As of now, the Center for Plant Resources has stored nearly 13,000 local rice accessions, including accessions from the CLRRI (http://csdl.prc.org.vn), and currently, the CLRRI genebank has also stored around 3000 local rice accessions collected from southern provinces of Vietnam (unpublished data). Among them, many precious genetic resources have been identified as aromatic rice (Tran Thi Luong et al. 2013), glutinous rice (Phan Huu Ton et al. 2013; Doan Thanh Quynh et al. 2016), pest-resistant rice (Barman et al. 2004; Tran Duy Cuong et al. 2012; Phan Huu Ton et al. 2013), and abiotic stress tolerance (Nguyen Thanh Tuong et al. 2005).

The local rice accessions have been utilized in the national rice breeding program to resolve food security and enhance the value of rice grains. Some of them include Nang Pha, Nang Tay, Tay Dum, Tau Binh, Chech Cut, and Bong Sen... cultivated currently in the floating rice system in An Giang and Dong Thap provinces of the Mekong Delta region (https://vnexpress.net/nong-dan-mien-tay-song-khoe-voi-lua-mua-noi-4519698.html), Nang Thom cho Dao in My Le commune, Can Duoc district, Long An province (https://dulichvietnam.com.vn/vi-sao-gao-nang-thom-cho-dao-long-an-tro-thanh-dac-san.html),

Nep Tam Xuan and Hom Rau cultivated in Quynh Phu District, Thai Binh Province (https://moit.gov.vn/tu-hao-hang-viet-nam/phat-trien-thuong-hieu-lua-gao-tinh-thai-binh-tren-kenh-phan-phoi-truyen-thong-va-thi-truong-thuong-mai-dien tu.html). For instance, local rice varieties were used as donors for improving disease resistance and tolerance to abiotic stresses as the use of the Tetep rice variety for increasing resistance to blast disease (Pita, Pik-h) in the rice breeding program, and OM11 and OM16 varieties were achievements of integrated polygenic resistance to blast disease (Tran Ngoc Thach et al. 2021).

Nearly 3000 local rice accessions of Vietnam have been stored in the seed bank of CLRRI so far. However, most of these have not undergone regeneration and re-evaluation for a long time. In addition, a part of the collection stored has no characters, and some important traits are not available in the Genebank database as traits related to abiotic stress tolerance and disease resistance. The complementing data for the Genebank database is in demand based on replanting experiments. Moreover, the regeneration and re-evaluation of local rice accessions will validate the conservation of CLRRI's Genebank and provide strategies for the exploitation of genetic resources in the future.

MATERIALS AND METHODS

Materials

The CLRRI's seed bank held 1,812 local rice accessions in total, of which a few samples were replanted in 2014 and nearly renewed in 2012. The 941 local rice varieties collected from Vietnam's central and southern provinces were imported from the International Rice Research Institute (IRRI), with 401 samples collected before 1975 through FAO.

Methods

Experimental design

Local rice seed samples were planted from August to December in the CLRRI field by transplanting at a distance of 25 x 30cm. Non-pesticides and a very low fertilizer dosage (20kg N/ha – 8kg P_2O_5/ha – 6kg K_2O/ha) were applied. The experiments were carried out by continuous design with an area of $10m^2$ for evaluating agronomic traits, $3m^2$ for rice seed multiplication, and $50m^2$ for evaluating promising accessions. Rice seeds were harvested, dried, and stored for medium-term and long-term conservation.

Package, storage, and management

The dried seed of each accession was cleaned, verified, and packaged in two ways as follows: rice seed was packed in 50g aluminum bags for long-term storage (-20^0C) and packed in 250g paper bags and wrapped in plastic bags (Ziploc bags) for medium-term storage (5^0C) (**Figure 1**). The data was managed in Excel files using Microsoft Excel software.

Figure 1. Packing and storing seed samples in long-term storage (left) and medium-term storage (right).

The agronomic traits for evaluating local rice accessions

Agronomic attributes consisted of Growth duration, Theoretical Yield, Plant Height, Panicle Length, Grain Length, Grain Width, Awn, Husk Color, Grain Shell Color, and Endosperm Chalk were measured and scored according to Standard Evaluation System for Rice (IRRI 2013). Data on the traits was analyzed using Microsoft Excel and Minitab software 16.2.0 (Minitab Inc. 2010).

RESULTS AND DISCUSSION

Regenerating local rice resources in 2018 and 2019

For the status of the CLRRI seed bank in 2018, a total of 1,812 local rice accessions were available in the CLRRI seed bank that was replanted in 2014 (**Figure 2A**). These accessions were planted to rejuvenate gene resources in the CLRRI experimental field in 2018 (**Figure 3**). The results indicated that 642 accessions were not germinated or germinated with small amount which meant that 272 accessions were only acquired with less than 50 g. The meager yield of harvested seed was mostly the result of low germination. Thus, a total of 1,170 accessions were verified and packaged in 50g aluminum bags for long-term storage while each of the 898 accessions weighing more than 250g was checked and packed in paper and plastic bags (Ziploc bags) (**Figure 2B**).

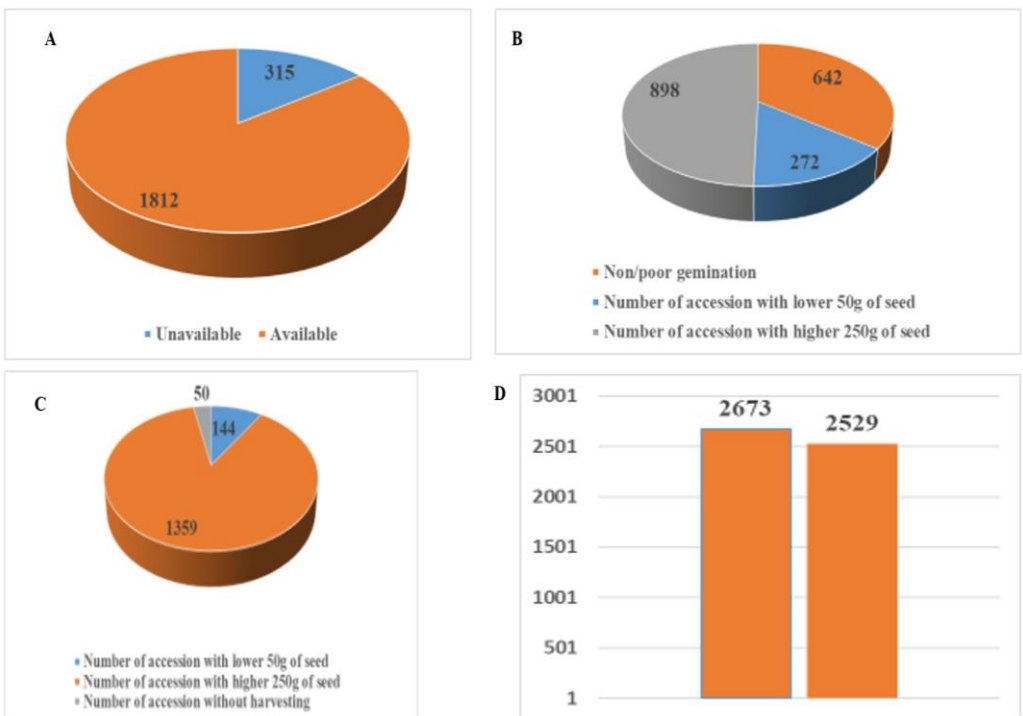

Figure 2. (A) Current situation of local rice resources in the CLRRI seed bank in 2018; **(B)** Germination of 1812 local rice accessions in 2018; **(C)** Results of regeneration of local rice accessions in 2019; **(D)** The number of accessions stored in two current medium- and long-term storages.

In 2019, 941 native Vietnamese local rice accessions imported from IRRI and 612 accessions with small amounts of seed from 2018 were replanted for rejuvenation and preliminary assessment of some basic characteristics. A total of 1,553 local rice

accessions were replanted in this crop, showing that, out of the total 1,503 samples harvested, 144 accessions had seed amounts less than 50g and 50 samples were not acquired due to photoperiod and other factors (**Figure 2C**). Thus, in total, the CLRRI seed bank held 2,673 local rice accessions as of 2019 (**Figure 2D**). In addition, 315 accessions that were absent in the seed bank should be re-collected through conservation initiatives in the future..

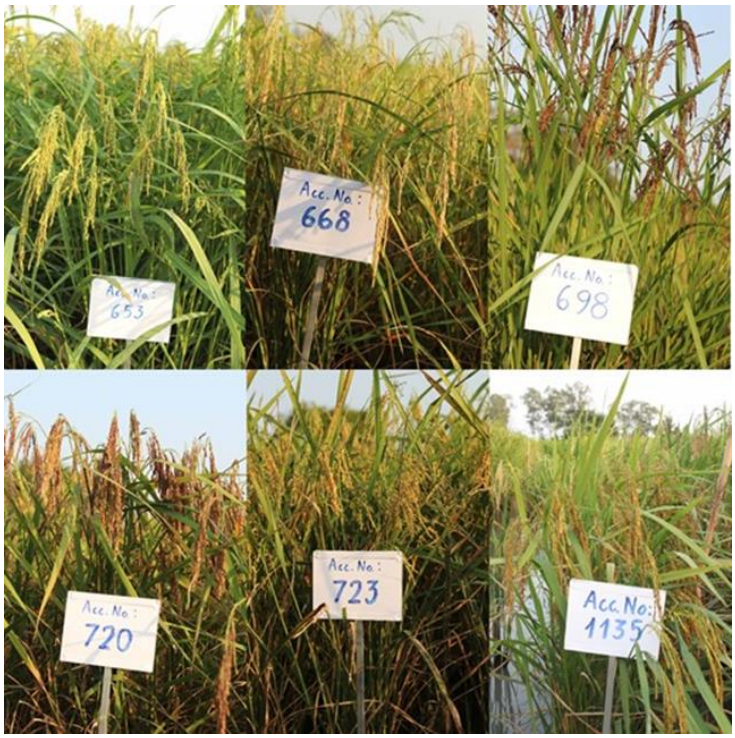

Figure 3. Some accessions in CLRRI experimental fields in 2018.

The diversity of local rice collection stored in the seed bank

In 2019, out of 612 accessions with a small amount of seed, 328 accessions were selected to evaluate some basic agronomic characteristics. The characteristics were grouped into quantitative traits (Growth duration, Theoretical Yield, Plant Height, Panicle Length, Grain Length, and Grain Width) and qualitative traits (Awn, Husk Color, Grain Shell Color, and Endosperm Chalk). The results showed that variations of these traits were significant in the study population (**Table 1**).

Table 1. Minimum (Min), maximum (Max) and mean values of some quantitative traits.

Traits	Min	Max	Mean
Growth duration (days)	97	140	111
Theoretical Yield (ton/ha)	1.7	10.3	5.3
Plant Height (cm)	110	170	122
Panicle Length (cm)	20	33	24.6
Grain Length (mm)	6.1	10.5	8.50
Grain Width (mm)	2	3.8	2.68

Histogram analysis showed that the distribution of local rice accessions in the population was centered around the mean of all quantitative traits (**Figure 4**). Accordingly, 319 accessions had a growth duration of 100-125 days (**Figure 4A**), 279 accessions had a theoretical yield of 3-8 t/ha (**Figure 4B**), 236 accessions had a plant height of 120-130 cm (**Figure 4C**), 314 accessions had a panicle length of 21-29 cm (**Figure 4D**), 319 accessions had a grain length of 7 – 10 mm (**Figure 4E**) and 321 accessions had a grain width of 2 – 3 mm (**Figure 4F**).

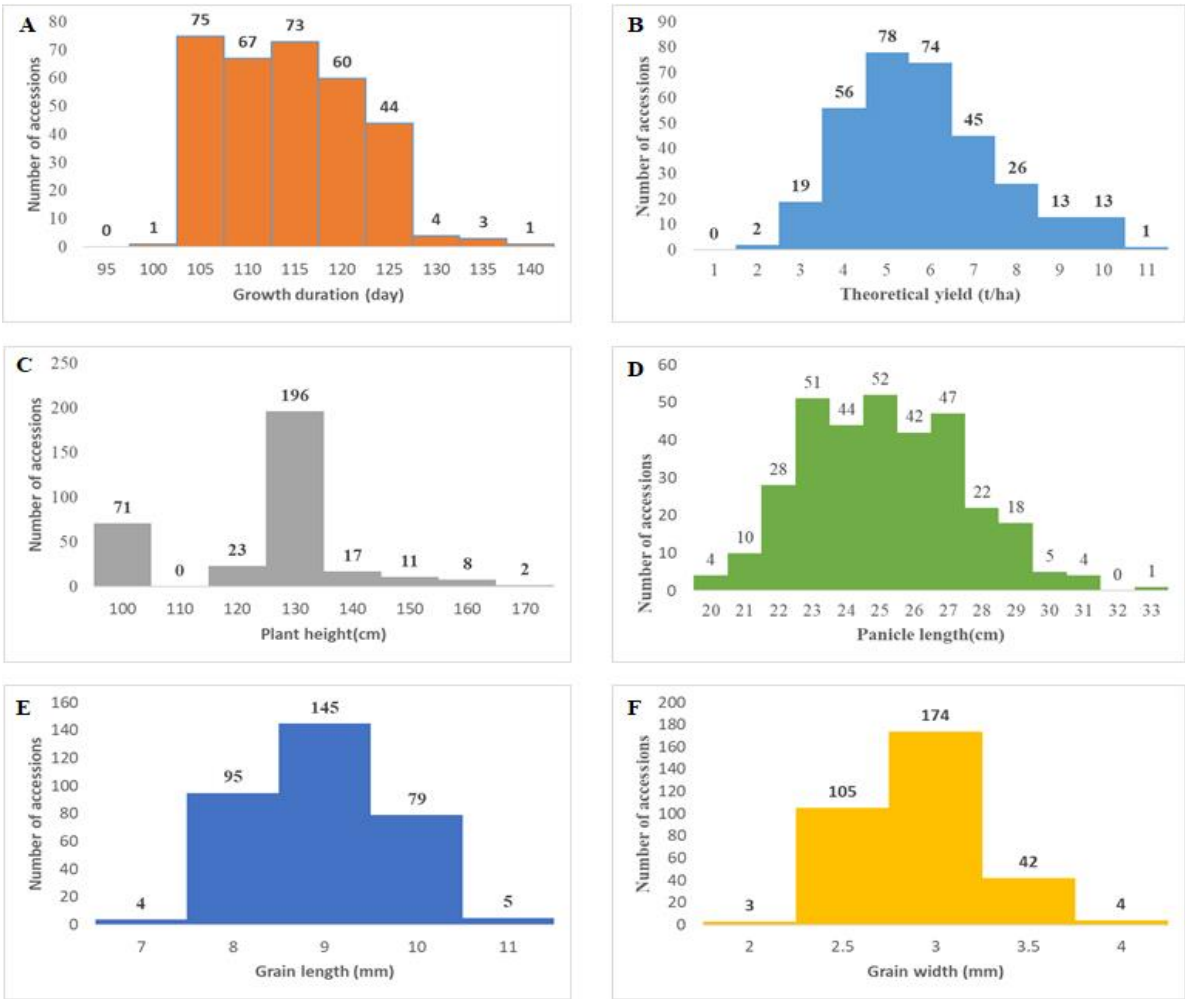

Figure 4. Distribution of accessions in the population based on quantitative traits, **(A)** Growth duration; **(B)** Theoretical yield; **(C)** Plant height; **(D)** Panicle Length; **(E)** Grain length; **(F)** Grain width.

For qualitative traits, only 35 accessions had awning character among investigated ones. The other traits, most accessions had straw-yellow lemma and palea color (**Fig. 5A**), white seed yellow lemma and palea color (**Fig. 5B**), and no chalkiness of endosperm (**Fig. 5C**). In addition, 19 accessions with opaque endosperm, including some varieties without glutinous rice names should be verified in other experiments. From variation of quantitative and qualitative traits in the population, the diversity of local rice accessions was significant.

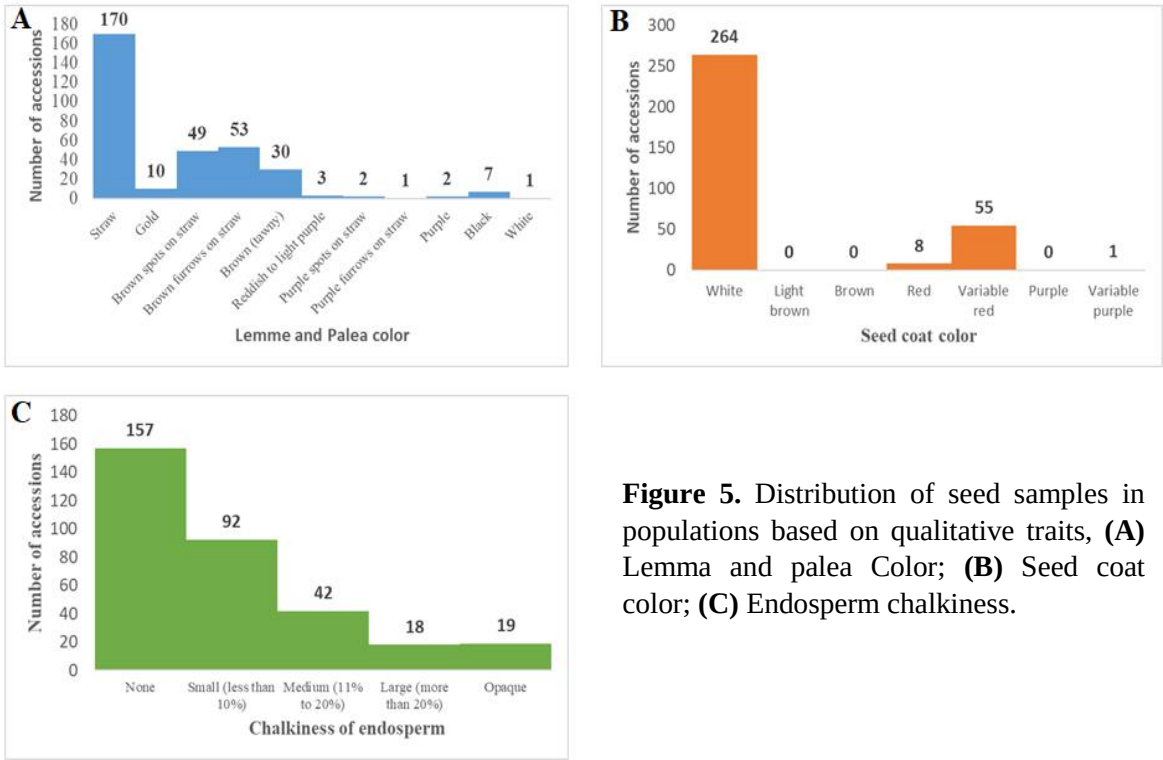

Figure 5. Distribution of seed samples in populations based on qualitative traits, **(A)** Lemma and palea Color; **(B)** Seed coat color; **(C)** Endosperm chalkiness.

Preliminary evaluation of local rice accessions imported from IRRI

In 2019, a total of 941 local rice accessions imported from IRRI, were planted for producing seed purposes. However, some other basic traits for the preliminary evaluation of this population were also observed. Traits were growth duration, theoretical yield, plant height, awn color, lemma and palea color, seed coat color, and endosperm opaque. The results showed that 38 accessions reacted to the photoperiod, and the growth duration of the 903 accessions ranged from 93 to 117 days. Of the 150 glutinous rice accessions, 142 accessions had completely opaque endosperm, and the remaining accessions had chalkiness of endosperm of level 5.

The accessions with the remaining traits distributed in the population were also analyzed, and the results of two quantitative traits found 855 accessions with theoretical yields of 3-6 t/ha and 800 accessions with a plant height of 80-140 cm (**Figure 7A, B**). For the qualitative traits of this population, most seed samples had no awning, yellow lemma and palea and white seed coat. However, 3 of 6 types of awning color, 9 of 11 types of lemma and palea color, and 7 types of seed coat color appeared in the population that reflected the diversity of local rice accessions in the population (**Figures 7C, D, E**).

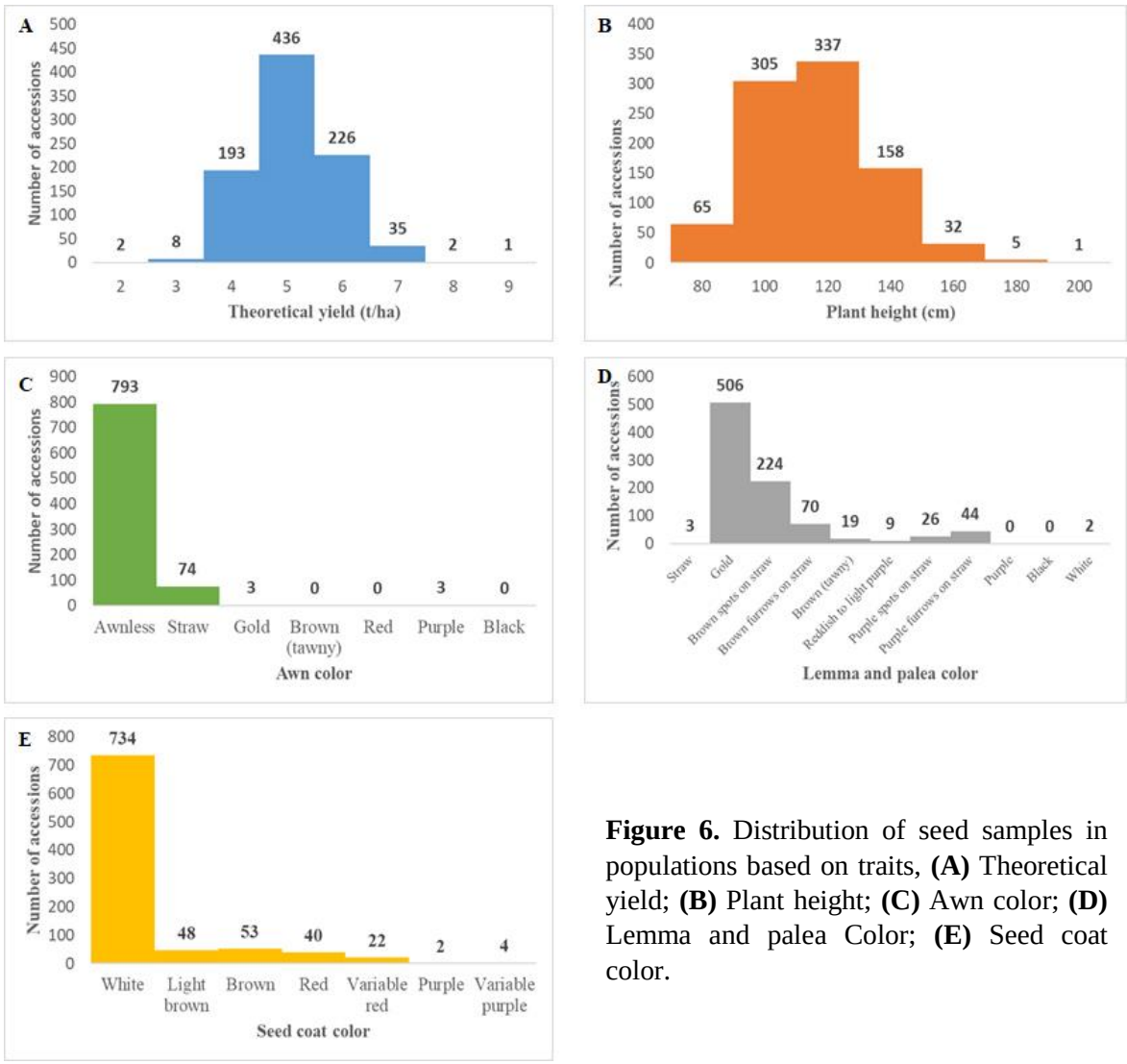

Figure 6. Distribution of seed samples in populations based on traits, **(A)** Theoretical yield; **(B)** Plant height; **(C)** Awn color; **(D)** Lemma and palea Color; **(E)** Seed coat color.

Identification of promising local rice accessions

Based on the evaluation in 2018, there were nine local rice accessions being selected among 1,812 local rice accessions investigated. These varieties were planted and evaluated with an area of 50m^2 per accession in 2019, the results in **Table 2** showed that the growth duration ranged from 105-115 days and yield from 3.4-4.4 tons/ha. Of those accessions 906-Nam Thom and 2014-Tau Huong with high yield, aromatic and soft rice are the potential for developing new varieties. The 1101-Nep Thngonh accession with long grain length could be a potential source for breeding long-grain glutinous rice. In addition, 1029-Tieu mo with Japonica rice-like shape should be validated and induced for breeding round seed rice (**Figure 7**).

Table 2. Evaluation results of 9 promising local rice accessions in 2019.

No.	Accession	Growth duration (days)	Hight Plant (cm)	Panicle Length (cm)	Grain Length (mm)	Grain Width (mm)	Chalk (level)	Yield (t/ha)	Aromatic	Cooked rice
1	737 -Nang Huong Cho Dao	110	152	24.3	8.9	2.4	5	3.6	non	-
2	906 -Nam Thom	105	150	25.3	10.2	2.3	5	4.4	slight	very soft
3	1015 -Tau Huong	113	155	25.5	8.1	2.3	9	3.8	non	-
4	1029 -Tieu mo	113	145	23.8	7.3	2.8	7	4.6	non	-
5	1101 -Nep Thngonh	115	128	26.5	10.0	1.9	opaque	3.4	non	-
6	1128 -Bat Ngat	115	143	25.3	9.3	2.0	7	3.8	non	-
7	1172 -HTA 87097	115	150	27.5	8.2	2.7	7	4.0	non	-
8	1408 -Nang Huong Cho Đao	115	153	28.2	9.0	2.5	5	3.6	non	-
9	2014 -Tau Huong	115	143	25.5	8.6	2.0	5	4.2	strong	very soft

906 – Nam Thom

2014 – Tau Huong

1101 – Nep Thngonh

1029 – Tieu mo

Figure 7. Seeds of some promising local rice accessions.

CONCLUSIONS

A total of 2,673 local rice accessions were stored in the CLRRI seed bank, of which 1,763 accessions were regenerated and 910 accessions were reproduced from the imported sources. A subset of 328 accessions was evaluated through 9 morphological traits, the results showed that the diversity of these accessions was significant. The preliminary evaluation of 903 imported local rice accessions also presented a high diversity. As a result of identifying promising accessions, two accessions were induced for developing quality rice, one for long-grain glutinous rice and another for round-grain rice.

COMPETING INTERESTS

The authors declare that they have no conflict of interest, financial or otherwise.

AUTHOR'S INFORMATION AND CONTRIBUTIONS

Nguyen Khac Thang conducted experiment and wrote the draft version of the manuscript. Tran Thu Thao conducted experiment, reviewed and edited the manuscript. Tran Binh Tan conducted experiment. Pham Cong Tru conducted experiment, reviewed and edited the manuscript. Nguyen Van Hieu conducted experiment, reviewed and edited the manuscript. Vo Thanh Toan conducted experiment, reviewed and edited the manuscript. Do Duc Tuyen designed the study, analyzed data, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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TRẺ HÓA NGUỒN GEN VÀ ĐÁNH GIÁ CÁC MẪU GIỐNG LÚA ĐỊA PHƯƠNG CỦA NGÂN HÀNG GEN VIỆN LÚA ĐỒNG BẰNG SÔNG CỬU LONG

Trong công tác bảo tồn nguồn gen, nhân nguồn trẻ hóa và đánh giá lại nguồn gen được xem là một bước không thể thiếu. Trước thực trạng ngân hàng gen hạt của Viện lúa Đồng bằng sông Cửu Long năm 2018, nhiều nguồn gen có nguy cơ bị mất. Với mục tiêu trẻ hóa và đánh giá lại các nguồn gen hiện có và nhập nội, nghiên cứu được tiến hành trên quần thể 1.812 mẫu giống lúa địa phương trong ngân hàng gen hạt và 941 mẫu giống lúa địa phương của Việt Nam nhập nội từ IRRI. Trong đó các mẫu giống được nhân nguồn và đánh giá trên ruộng thí nghiệm trong các vụ mùa 2018 và 2019. Kết quả thu được là 2.673 mẫu giống lúa địa phương được nhân nguồn trẻ hóa và lưu giữ trở lại ngân hàng gen hạt. Trong đó hai quần thể 328 và 903 mẫu giống lúa địa phương được đánh giá và đánh giá sơ bộ một số tính trạng, kết quả cho thấy sự đa dạng đáng kể của hai quần thể này. Các mẫu giống lúa địa phương triển vọng cũng được đánh giá và giới thiệu được 4 mẫu giống cho mục đích khai thác nguồn gen chất lượng, nếp hạt dài và hạt tròn. Với những kết quả đạt được rất có thể là tiền đề cho công tác bảo tồn và khai thác nguồn gen của Viện trong tương lai.

Từ khóa: *Bảo tồn nguồn gen, lúa địa phương, nhân nguồn trẻ hóa nguồn gen.*